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Speradine A, a new pentacyclic oxindole alkaloid from a marine-derived fungus *Aspergillus tamarii*

Masashi Tsuda,[a] Takao Mugishima,[a] Kazusei Komatsu,[a] Teruo Sone,[b] Michiko Tanaka,[b] Yuzuru Mikami,[c] Motoo Shiro,[d] Manabu Hirai,[e] Yasushi Ohizumi[e] and Jun'ichi Kobayashi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita-12 Nishi-6, Kita-ku, Sapporo 0600812, Japan*
[b]*Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan*
[c]*Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan*
[d]*X-Ray Research Laboratory, Rigaku Corporation, Akishima, Tokyo 196-8666, Japan*
[e]*Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-0845, Japan*

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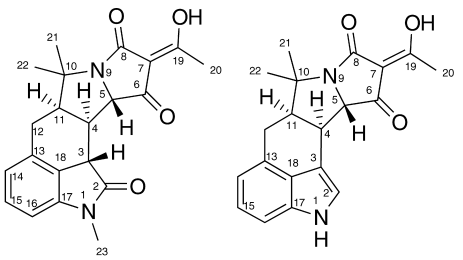
Abstract—A new pentacyclic oxindole alkaloid, speradine A (**1**), was isolated from the cultured broth of a fungus *Aspergillus tamarii*, which was separated from driftwood at a seashore in Okinawa. The structure and relative stereochemistry were determined by spectroscopic data and a single crystal X-ray diffraction analysis.

1. Introduction

Marine-derived fungi have proven to be a rich source of structurally novel and biologically active secondary metabolites.[1] In our search for new substances from marine-derived fungi,[2] a new pentacyclic oxindole alkaloid, speradine A (**1**), was isolated from the cultured broth of a fungus *Aspergillus tamarii*, which was separated from driftwood at a seashore in Okinawa. In this paper we describe the isolation and structure elucidation of **1**.



1 **2**

2. Results and discussion

The fungus *A. tamarii* (strain M143) was separated from driftwood collected at Seragaki Beach, Okinawa Island, and grown in Czapek-Dox liquid medium containing 3% peptone for 14 days at 28°C. The supernatant of the culture broth (2 L) was extracted with EtOAc, and the EtOAc-soluble portions were subjected to silica gel column chromatography and then C_{18} HPLC to afford speradine A (**1**) together with a known related alkaloid, cyclopiazonic acid[3–5] (**2**).

Speradine A {**1**, $[\alpha]_D^{18}=-79°$ (*c* 1.0, $CHCl_3$)} had the molecular formula of $C_{21}H_{22}N_2O_4$ as revealed by HRFABMS [*m/z* 367.1644, $(M+H)^+$, Δ −1.4 mmu]. IR absorption bands at 3423 and 1719 cm^{-1} were attributed to OH/NH and carbonyl group(s), respectively. The UV absorption at 280 nm (ε 6400) was suggestive of the presence of a benzenoid chromophore. ^1H and ^{13}C NMR data (Table 1) disclosed the existence of eight sp^2 quaternary carbons including four relatively low-field ones (δ_C 193.13, 184.42, 177.56, and 173.20), three sp^2 methines, one sp^3 quaternary carbon, four sp^3 methines, one sp^3 methylene, and four methyl groups, one (δ_H 3.18, δ_C 26.44) of which was attached to a nitrogen atom. Three lower-field proton signals at δ_H 6.67 (d, *J*=7.5 Hz), 6.86 (d, *J*=7.6 Hz), and 7.20 (t, *J*=7.5 Hz) indicated the presence of a 1,2,3-trisubstituted benzene ring. ^1H and ^{13}C NMR data of **1** were similar to those of cyclopiazonic acid[3–5] (**2**), except for the chemical shifts of C-2 (δ_C 177.56, s) and C-3 (δ_C 43.38, d) and the existence of an *N*-methyl group (C-23) in **1**. The $^1H-^1H$ COSY and HMQC spectra revealed connectivities from C-14 to C-16 and from C-3 to C-5 and C-12 (Fig. 1).

Keywords: speradine A; oxindole alkaloid; *Aspergillus tamarii*.
* Corresponding author. Tel.: +81-11-706-3922; fax: +81-11-706-4989; e-mail: jkobay@pharm.hokudai.ac.jp

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Table 1. ^{1}H, ^{13}C, and ^{15}N NMR data of speradine A (**1**) in $CDCl_3$

Position	$\delta_{C(N)}$	m	δ_H	(m, Hz)	HMBC (H)
1	128.8[a]				23
2	177.56	s			3, 4, 23
3	43.38	d	3.48	d, 6.8	4, 5
4	38.15	d	2.54	m	3, 5, 12a,[b] 12b
5	71.43	d	4.27	d, 9.3	3, 4
6	193.13	s			4, 5
7	104.69	s			20
8	173.20	s			5
9	134.3[a]				21, 22
10	62.45	s			21, 22
11	55.10	d	2.17	m	12b, 21, 22
12	27.72	t	2.76	dd, 5.6, 14.1	14
			2.63	brt, 12.6	
13	134.97	s			12a, 12b, 15
14	120.29	d	6.86	d, 7.6	16
15	128.42	d	7.20	t, 7.5	
16	105.87	d	6.67	d, 7.5	14
17	142.74	s			15, 23
18	126.09	s			3, 12a, 14, 16
19	184.42	s			20
20	19.49	q	2.43[c]		
21	24.73	q	1.50[c]	s	22
22	26.29	q	1.61[c]	s	21
23	26.44	q	3.18[c]	s	

[a] The ^{15}N chemical shifts were assigned by $^{1}H-^{15}N$ HMBC spectrum.
[b] a and b denote lower-field and higher-field resonances, respectively, of a geminal pair for C-12.
[c] 3H.

Signals of two nitrogens observed at δ_N 128.8 (N-1) and 134.3 (N-9) were assigned by $^{1}H-^{15}N$ HMBC correlations from H_3-23 and H_3-21 (H_3-22), respectively. Analysis of $^{1}H-^{13}C$ HMBC correlations suggested the presence of a tetracyclic ring system in **1** consisting of rings A, C, D, and E like **2**. Since the carbon chemical shifts of C-6, C-7, C-8, C-19, and C-20 were close to those of **2**, the geometry of the C-7–C-19 double bond in **1** was assigned as the same as that

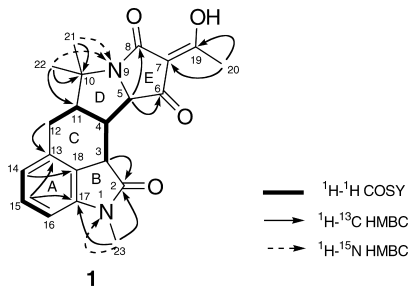

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Figure 1. Selected 2D NMR correlations for speradine A (**1**).

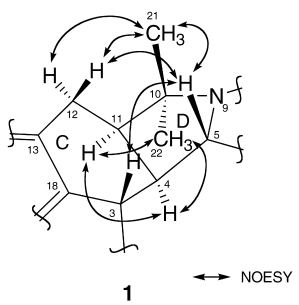

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Figure 2. NOESY correlations and relative stereochemistry for C and D rings of speradine A (**1**).

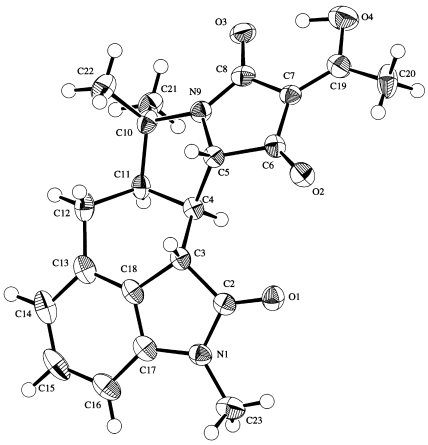

Figure 3. Molecular structure of speradine A (**1**) obtained by X-ray analysis (ORTEP drawing; ellipsoids are drawn at 30% probability level).

of **2**, which was considered to be an equilibrium mixture of the four possible enolic tautomers with the *Z-exo*-enol form predominating.[6,7] HMBC correlations for H_3-23 to C-2 and C-17 and H-3 to C-2 indicated the presence of a 1-*N*-methyl-2-oxindole ring. Thus, the gross structure of speradine A was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from $^{1}H-^{1}H$ coupling constants (Table 1) and NOESY correlations (Fig. 2). *anti*-Relations for H-3 to H-4 and H-4 to H-5 were deduced from *J*(H-3/H-4) (6.8 Hz) and *J*(H-4/H-5) (9.5 Hz) values, respectively, while the relation between H-4 and H-11 was indicated to be *syn* by the NOESY correlation for H-4/H-11. Ring C was implied to have a pseudo-boat form by NOESY correlations as shown in Figure 2. Therefore, the relative stereochemistry of speradine A was assigned as **1**.

Speradine A (**1**) was crystallized from hexane–$CHCl_3$–MeOH as yellow platelets, mp 145–147°C. The relative stereochemistry of the 4 chiral centers in **1** was confirmed by a single crystal X-ray diffraction analysis, and the perspective view of the final X-ray model is shown in Figure 3. An intramolecular hydrogen bond (1.82 Å) was observed between a hydroxyl proton (O4) and an amide carbonyl oxygen atom (O3).

Speradine A (**1**) with a 1-*N*-methyl-2-oxindole ring is a new congener of cyclopiazonic acid (**2**). Speradine A (**1**) exhibited inhibitory activity against Ca^{2+}-ATPase (IC_{50} 8 μM), inhibitory activity against histone deacetylase (IC_{50} 100 μg/mL), and antibacterial activity against *Mycrococcus luteus* (MIC 16.7 μg/mL).

3. Experimental

3.1. General experimental procedures

Melting point was determined with a Yanaco micro melting point apparatus and was uncorrected. Optical rotation was measured on a JASCO DIP-1000 polarimeter. The IR and UV spectra were recorded on a JASCO FT/IR-5300 and a Shimadzu UV-1600PC spectrophotometer, respectively. CD spectra were measured on a JASCO J-720

spectropolarimeter. NMR spectra were recorded on a Bruker AMX-600 spectrometer. FAB mass spectrum was obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix. For $^{1}H-^{15}N$ HMBC experiments, 95% formamide in $CDCl_3$ was used for external reference (δ_N 112.4) of ^{15}N NMR. The spectral width in F_1 of $^{1}H-^{15}N$ HMBC was 50–150 ppm, and a total of 128 increments of 1*K* data points were collected. $^{1}H-^{15}N$ HMBC was measured using a 50 ms delay for long range N–H coupling.

3.2. Fungal material and fermentation

The fungus *A. tamarii* (M143) was separated from driftwood, which was collected at Seragaki Beach, Okinawa Island. From the sequence analysis of the 18 S rDNA and ITS region including the 5.8 S rDNA, this strain was found to be similar to *A. tamarii* (similarity, 100%/1733 bps, based on 18 S rDNA, 99.7%/580 bps, based on the ITS region). According to both morphological and molecular biological data, this fungus was identified as *A. tamarii*. The sequence data of this strain M143 have been submitted to the DDBJ/EMBL/GenBank under accession No. AB106338 and AB106339. Subcultures of the organism were deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University. The fungus was grown in Czapek-Dox liquid medium containing 3% peptone ($NaNO_3$, 3 g; K_2HPO_4, 1 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4{\cdot}7H_2O$; 0.01 g; sucrose, 30 g; peptone, 30 g; seawater, 500 mL; distilled water, 500 mL) for 14 days at 28°C. The cultured broth (2 L) was filtered.

3.3. Extraction and separation

The filtrate of the cultured broth (2 L) was extracted with EtOAc (1 L×2). The EtOAc-soluble portions (377 mg) were subjected to silica gel column chromatography ($CHCl_3$/MeOH, 99:1 and then $CHCl_3$/EtOH, 99:1) followed by C_{18} HPLC [YMC-Pack Hydrosphere C18, YMC Co., Ltd, 10×250 mm: flow rate 2.5 mL/min; UV detection at 281 nm; eluent: $CH_3CN/H_2O/CF_3CO_2H$, 65:35:0.1) to give speradine A (**1**, 12 mg) and cyclopiazonic acid (**2**, 17 mg). **2**: colorless amorphous solid; $[\alpha]_D^{18}=-74°$ (*c* 1.0, $CHCl_3$); CD (MeOH) λ_{ext} 310 ($\Delta\varepsilon$ +1.1), 280 (±0), 268 (−5.0), 251 (−1.0), 226 (−25.6), 212 (±0), and 204 (+17.6).

3.3.1. Speradine A (1). Pale yellow solid; $[\alpha]_D^{18}=-79°$ (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 280 (ε 6400) 253 (7700) and 210 (15000) nm; CD (MeOH) λ_{ext} 305 ($\Delta\varepsilon$ +2.4), 286 (±0), 262 (−11), 232 (±0), 230 (+0.2), 229 (±0), 217 (−9.4), and 209 (±0); IR (KBr) ν_{max} 3423, 2925, 1719, 1611, and 1475 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS *m/z* 367 $(M+H)^+$; HRFABMS *m/z* 367.1644 $(M+H)^+$ (calcd for $C_{21}H_{23}N_2O_4$, 367.1658).

3.4. X-Ray analysis of speradine A (1)

Speradine A (**1**) was crystallized from hexane–$CHCl_3$–MeOH as yellow platelets, mp 145–147°C. Crystal data: $C_{21}H_{22}N_2O_4$, M_r=366.42, crystal dimensions 0.25×0.15×0.03 mm, orthorhombic, space group $P2_12_12_1$ (no. 19), *a*=7.338(1) Å, *b*=10.661(1) Å, *c*=22.348(3) Å, *V*=1748,2(4) $Å^3$, *Z*=4, D_{calc}=1.392 g/cm^{-1}. A crystal was coated with liquid paraffin. All measurements were made on Rigaku RAXIS-RAPID Imaging Plate diffractometer with graphite monochromated Mo Kα radiation (λ=0.71075 Å) at a temperature of −180±1°C to a maximum 2θ value of 60.1°. A total of 55 images, corresponding to 220.0° oscillation angles, were collected with two different goniometer settings. Exposure time was 1.00 min per degree. The camera radius was 127.40 mm. Readout was performed in the 0.100 mm pixel mode. Data were processed by the PROCESS-AUTO program package. Of the 20403 reflections which were collected, 2915 were unique (R_{int}=5.5%); equivalent reflections were merged. The linear absorption coefficient, μ, for Mo Kα radiation was 1.0 cm^{-1}. An absorption correction was not applied. The data were corrected for Lorentz and polarization effects.

The structure was solved by direct methods (SIR97)[8] and expanded using the Fourier technique (DIRDIF94).[9] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full matrix least-squares refinement was based on 2915 observed reflections ($I>-3.00\sigma(I)$, $2\theta<60.06$) and 247 variable parameters and converged with unweighed and weighed agreement factors of R=0.082, R_w=0.076. The standard deviation of an observation of unit weight was 1.15. The weighting scheme was based on counting statistics and included a factor (p=0.018) to downweight the intense reflections. Plots of $\omega(F_0^2-F_c^2)^2$ versus F_0^2, reflection order in data collection, $\sin\theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.54 and −0.53 $e^-/Å^3$, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation. Crystallographic data for speradine A (**1**) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 198230).

Acknowledgements

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